ERCC1 and *BRAC1* mRNA Expression Levels in the Primary Tumor Could Predict the Effectiveness of the Second-Line Cisplatin-Based Chemotherapy in Pretreated Patients with Metastatic Non-small Cell Lung Cancer

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Introduction: The potential predictive role of BRCA1 and ERCC1 expression levels in patients with metastatic non-small cell lung cancer (NSCLC) receiving second-line platinum-based chemotherapy was investigated.

Methods: Real-time quantitative polymerase chain reaction after reverse transcription was used to assess the expression levels of BRCA1 and ERCC1 in 100 microdissected primary tumors from platinum-naive NSCLC patients treated with platinum-based chemotherapy in the second-line setting.

Results: Low *ERCC1* mRNA levels were significantly associated with higher response rate ($p = 0.011$), longer median progression-free survival (PFS; $p = 0.029$), and median overall survival (mOS; $p = 0.001$) after the initiation of the second-line treatment. Similarly, low *BRCA1* expression level was significantly correlated with higher response rate ($p = 0.022$), longer PFS ($p = 0.041$), and mOS ($p = 0.005$). In addition, patients with low *ERCC1* and *BRCA1* mRNA experienced increased median PFS ($p = 0.021$) and mOS ($p < 0.001$) in comparison with those who had both genes upregulated. A multivariate analysis revealed that low *ERCC1* and low *BRCA1* expression levels were significantly associated with increased PFS (hazard ratio [HR]: 0.6; 95% confidence interval [CI]: 0.4–0.8; $p = 0.029$ and HR: 0.7; 95% CI: 0.6–0.9; $p = 0.043$, respectively) and OS (HR: 0.5; 95% CI: 0.3–0.7; $p = 0.003$ and HR: 0.7; 95% CI: 0.6–0.9; $p = 0.038$, respectively).

Conclusions: These results suggest that the *ERCC1* and *BRCA1* mRNA expression levels in the primary tumor at the time of diagnosis could be used for the prediction of platinum sensitivity in the treatment of NSCLC in the second-line setting. Cross-validation studies are warranted.

Key Words: BRCA1, ERCC1, NSCLC, Cisplatin, Second-line chemotherapy.

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer death among men and women worldwide, and almost 70% of them have locally advanced or metastatic disease at diagnosis.[1] A combination of platinum compounds with third-generation agents (vinorelbine, gemcitabine, docetaxel, and paclitaxel) has become the standard of palliative care for patients with stage IIIB/IV NSCLC and good performance status (PS) and has significantly improved median survival and quality of life of these patients.[2,3] Alternatively, chemotherapy regimens that do not contain platinum doublets have also been tested in several randomized phase III studies with substantial efficacy and a more favorable toxicity profile in advanced/metastatic NSCLC patients.[4–6] Despite these advances, most patients eventually relapse or become refractory to first-line chemotherapy generally within a median of 3 to 6 months from the initiation of treatment. Docetaxel, pemetrexed, and erlotinib have been approved as the current options of second-line treatment for advanced NSCLC patients with significant survival benefit and improvement of quality of life.[7–10] However, the role of platinum efficacy in second-line treatment of NSCLC patients is not well defined because the majority of patients receive platinum compounds in the first-line setting. A few randomized phase II studies have evaluated the efficacy of platinum combinations in second-line therapy in either platinum-pretreated or platinum-naive NSCLC patients.[11–13] Nevertheless, the results from the second-line treatment combinations are disappointing with median survival of 8 months and 30% for 1-year survival.[11]

Over the last decade, data from gene expression, mutational, and proteomic profiling studies as well as from in vitro models led to the identification of molecular markers

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that could influence the treatment decisions in the daily clinical practice. A growing body of evidence is emerging for excision repair complementation group 1 (ERCC1) and breast cancer susceptibility gene 1 (BRCA1) and their role as potential predictors for platinum-based chemotherapy. ERCC1 is a rate-limiting enzyme in the nucleotide excision repair (NER) pathway, which recognizes and removes platinum adducts and repairs inter- and intrastrand cross-links.[14] ERCC1 overexpression is associated with cellular resistance to platinum compounds, whereas downregulation of ERCC1 sensitizes cells to cisplatin.[15,16] ERCC1 has been evaluated as a prognostic[17] and predictive[18–20] biomarker for response and survival in NSCLC patients. Its predictive significance has also been shown in biopsies of ovarian[21] and colorectal[22] cancer patients.

Apart from its role in transcriptional regulation, cell cycle control, ubiquitination, apoptosis, and mitotic spindle assembly, BRCA1 has a prominent role in DNA repair and regulation of genome stability.[23] BRCA1 is involved in platinum adducts removal as a component of transcription-coupled NER and homologous recombination repair pathways during the repair of double-strand breaks.[24,25] Experimental[26,27] and clinical[28,29] studies have demonstrated that *BRCA1* mRNA expression level is differentially associated with the response to chemotherapeutic drugs and ionizing irradiation. Low *BRCA1* mRNA is correlated with cisplatin sensitivity and taxane resistance. The above-mentioned studies suggested that both *BRCA1* and *ERCC1* mRNA expression levels could predict resistance when platinum compounds were administered in an adjuvant[17,20,30] or first-line setting.[5,6,19,31,32,] However, it is still unknown whether the expression levels of *BRCA1* and *ERCC1* in the primary tumor could predict NSCLC patient's outcome when treated with second-line platinum-based chemotherapy. In a previous retrospective study, we have observed that advanced NSCLC patients with low levels of *BRCA1* who had been treated with a docetaxel/gemcitabine regimen in the first-line setting, obtained the maximum benefit from cisplatin-based second-line chemotherapy.[33] To confirm this observation, we investigated the relevance of this finding in an independent group of 100 additional patients by evaluating the role of *BRCA1* and *ERCC1* expression levels as potential predictive markers for platinum-based second-line chemotherapy.

PATIENTS AND METHODS

Patients

Samples from primary tumors of NSCLC patients with histologically confirmed stage IV, who received a platinum-based regimen as second-line treatment, were retrospectively collected and analyzed. These patients were selected from a cohort treated with nonplatinum-containing regimen as first-line treatment, in the context of two randomized trials in which a nonplatinum doublet was compared with a platinum regimen (docetaxel + cisplatin versus docetaxel + gemcitabine[5] and vinorelbine + cisplatin versus docetaxel gemcitabine[6]) of the Hellenic Oncology Research Group.[5,6] After disease progression, the patients treated with nonplatinum doublet received cisplatin alone or a cisplatin combination regimen as second-line treatment in the context of a prospective randomized trial.[11,12] The main eligibility criteria have been previously reported.[5] The study has been approved by the institutional ethics and scientific committees and was conducted according to the Declaration of Helsinki. All patients signed a written consent form at the time of their initial evaluation both for the participation in the trial of frontline chemotherapy and for the use of their tissue for translational research. All laboratory analyses were performed blinded to the clinical data.

Specimens' Characteristics and Assay Methods

Formalin-fixed paraffin embedded tumors were reviewed by two independent pathologists (E.L. and E.S.) to select the most appropriate area for microdissection. Serial sections of 5 μm were prepared and stained with nuclear Fast Red (Sigma–Aldrich, St. Louis, MO). An Eppendorf piezoelectric microdissector (Eppendorf, Hamburg, Germany) was used to procure only malignant cells. RNA extraction from microdissected cells, reverse transcription, reverse-transcriptase polymerase chain reaction (RT-PCR) were performed as previously described.[34] The primers and probe sets have been previously reported.[35]

Relative cDNA quantification was performed using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Carlsbad, CA). Comparative C_t method was used for gene expression quantification using *β-actin* and *PGK1* as internal reference genes and commercial RNA (Stratagene, La Jolla, CA) as calibrators. Final expression values were determined as follows: $2^{-(\Delta Ct\ sample - \Delta Ct\ calibrator)}$, where ΔC_t values of the sample and

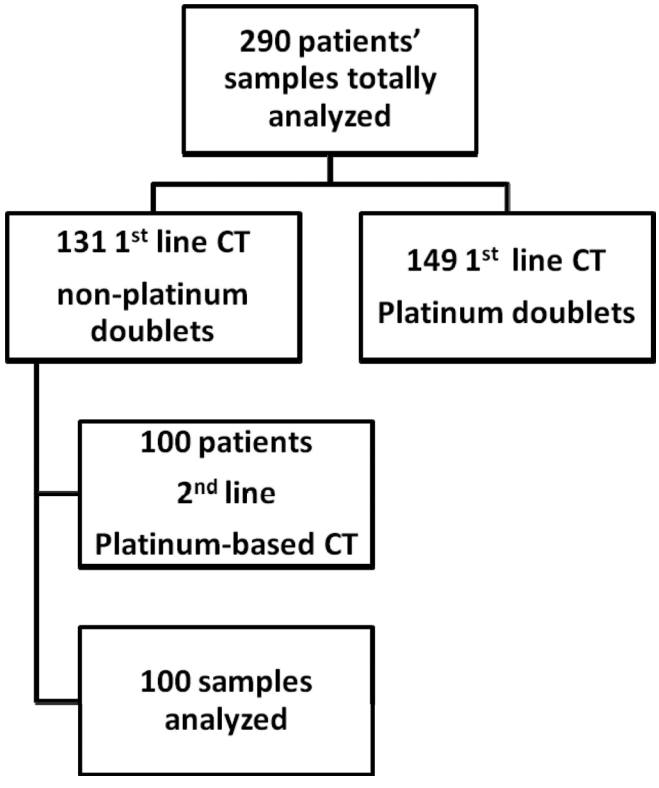

FIGURE 1. Flow chart of the study.

TABLE 1. Patients' Characteristics (N = 100)

Feature	*n*	Percentage
Median age (range)		63 (34–78)
≤70 yr	74	74
>70 yr	26	26
Gender		
Male	79	79
Female	21	21
Tumor histology		
Squamous	23	23
Nonsquamous	77	77
ECOG-PS		
0	26	26
1	74	74
First-line regimens		
Docetaxel/gemcitabine	71	71
Vinorelbine/gemcitabine	29	29
Response to first-line treatment		
CR + PR	38	38
SD + PD	62	62
Second-line treatment		
Cisplatin + irinotecan	34	34
Cisplatin	51	51
Cisplatin + pemetrexed	15	15
ERCC1 mRNA expression level		
Squamous (median, 3.89)	23	23
High	13	13
Low	10	10
Nonsquamous (median, 2.86)	77	77
High	42	42
Low	35	35
All histologies		
High	55	55
Low	45	45
Treatment group		
Cisplatin + irinotecan		Median, 2.93
High	19	19
Low	15	15
Cisplatin		Median, 2.88
High	27	27
Low	24	24
Cisplatin + pemetrexed		Median, 2.81
High	9	9
Low	6	6
BRCA1 mRNA expression levels		
Squamous (median, 8.10)	23	23
High	14	14
Low	9	19
Nonsquamous (median, 3.62)	77	77
High	40	40
Low	37	37
All histologies		
High	54	54
Low	46	46
Treatment group		
Cisplatin + irinotecan		Median, 4.85
High	18	18
Low	16	16
Cisplatin		Median, 4.69
High	29	29
Low	22	22
Cisplatin + pemetrexed		Median, 4.63
High	9	9
Low	6	6

ECOG, Eastern Cooperative Oncology Group; PS, performance status; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

the calibrators are estimated by subtracting the C_t value of the target gene from the median values of the housekeeping genes. In all experiments, only triplicates with a standard deviation of the C_t value less than 0.25 were accepted. In addition, genomic DNA contamination was excluded by including nonreverse-transcribed RNA as a control for all 100 patients' samples.

Study Design and Statistics

In this retrospective study, we aim to explore the predictive significance of *BRCA1* and *ERCC1* mRNA expression levels in the primary tumor of NSCLC platinum-naive patients treated with platinum-based second-line chemotherapy. All available biopsies of the primary tumor with more than 100 cells per section were analyzed. Objective responses were recorded according to the RECIST criteria.[36] All efficacy results were assessed on an intention-to-treat basis. Median progression-free survival (PFS) and median overall survival (mOS) were calculated from the start of second-line treatment to the documented disease progression or death, respectively. A quantitative PCR analysis yielded values that were expressed as ratios between two absolute measurements (target gene:reference genes). Cutoff points were predefined and reported from previous investigations conducted in our laboratory.[32]

Correlations between treatment outcome parameters and mRNA expression levels were assessed using Fisher's exact test. Kaplan–Meier curves were used to plot the corresponding time-to-progression and survival curves. A univariate Cox regression analysis, with hazard ratios (HRs) and 95% confidence intervals (CIs), was used to determine the association between each prognostic factor and clinical parameter. These factors were then included in a multivariate Cox proportional hazards regression model with a stepwise procedure (both forward and backward) to evaluate the independent significance of different variables on survival and time to progression. Statistical significance was set at $p = 0.05$.

RESULTS

Patients' Characteristics and mRNA Expression Levels

In total, 414 NSCLC patients were treated with docetaxel plus gemcitabine in the context of two randomized

trials contacted by Hellenic Oncology Research Group.[5,6] In total, 290 representative samples from the primary tumors of these patients were analyzed in the Laboratory of Tumor Cell Biology since 2006. Full clinical data, including patients' management after progression to first-line chemotherapy, were available for all patients. From the initial 290 patients, 131 were treated with a nonplatinum-containing doublet in the context of two randomized clinical trials.[5,6] One hundred of these 131 platinum-naive patients had received cisplatin-based chemotherapy as second-line treatment (Figure 1).[11,12] Patients' characteristics were typical for NSCLC and are summarized in Table 1. In an intention-to-treat analysis, partial response was observed in 14 (14%) patients (overall response rate [ORR] 14%; 95% CI: 8.2–23.6). After a median follow-up period of 9.9 months (range, 1.2–62.6 months), the median PFS was 3.3 months (95% CI: 1.9–4.6), and the mOS was 8.9 months (95% CI: 7.4–12.7). These results are comparable with those reported for the whole population of the two studies.[11,12]

The cutoff points for the expression values of *BRCA1* and *ERCC1* were previously predefined in our laboratory and were different for squamous and nonsquamous tumor, because the mRNA expression levels for *BRCA1* and *ERCC1* recorded in squamous cell carcinomas were significantly higher than those of adenocarcinomas ($p = 0.001$ for *BRCA1* and $p = 0.03$ for *ERCC1*, respectively), as previously reported.[32,37] Furthermore, a significant correlation between *BRCA1* and *ERCC1* mRNA expression levels (Spearman's test 0.39; $p = 0.002$) was observed. Using these predefined cutoff values, low (below the cutoff) tumoral *BRCA1* expression level was observed in 46 patients (46%), whereas low expression level of *ERCC1* was observed in 45 patients (45%) (Table 1). The *ERCC1* and *BRCA1* mRNA expression levels were similar across the three treatment groups with no statistical difference between them as shown in Table 1.

Genes' Expression Levels and Response to Second-Line Cisplatin-Based Treatment

The correlations between response to treatment, PFS and mOS, and *BRCA1* and *ERCC1* mRNA expression levels are summarized in Table 2. Patients with high *BRCA1* mRNA expression level had significantly lower ORR (6% versus 26%; $p = 0.034$) and decreased PFS (2.2 versus 4.0 months; $p = 0.041$; Figure 2*A*) and mOS (6.7 versus 14.7 months; $p = 0.005$; Figure 3*A*) to second-line cisplatin-based chemotherapy in comparison with those with low *BRCA1* mRNA levels. Similarly, patients with high *ERCC1* mRNA expression level, when they were treated with cisplatin-based chemotherapy in the second-line setting, experienced a lower ORR (7% versus 29%, $p = 0.013$) and a shorter PFS (2.0 versus 4.2 months; $p = 0.003$; Figure 2*B*) and mOS (5.8 versus 15.8 months; $p = 0.001$; Figure 3*B*), as compared with patients whose tumors had low *ERCC1* mRNA expression level. Moreover, second-line treatment with cisplatin-based chemotherapy is significantly less efficacious in patients with overexpression of both *BRCA1* and *ERCC1* in their primary tumors in terms of ORR (4% versus 30%; $p = 0.006$), PFS (2.0 versus 4.1 months; $p = 0.002$; Figure 2*C*), and mOS (5.4 versus 16 months; $p = 0.001$; Figure 3*C*) than in those patients with low mRNA expression level in their primary tumors. Finally, patients with low mRNA expression level of both *BRCA1* and *ERCC1* genes presented significantly higher ORR (30% versus 8%; $p = 0.035$), increased mOS (16 versus 8.8 months; $p = 0.008$; Figure 3*C*), and a statistical trend toward a longer PFS (4.1 versus 3.4 months; $p = 0.054$; Figure 2*C*), as compared with patients with high mRNA expression level of either *BRCA1* or *ERCC1* genes.

Univariate and Multivariate Analyses

A univariate analysis demonstrated that high *BRCA1* (HR: 1.61, 95% CI: 1.20–2.83; $p = 0.002$) and *ERCC1* (HR: 1.82, 95% CI: 1.37–3.13; $p = 0.001$) mRNA expression level, Eastern Cooperative Oncology Group–PS of 1 (HR: 1.56, 95% CI: 1.29–2.58; $p = 0.026$), and no response to

TABLE 2. mRNA Expression Levels of *BRCA1* and *ERCC1* and Treatment Efficacy

Genes mRNA Expression Level	No. of Patients	PFS (mo) Median (95% CI)	PFS (mo) p^a	OS (mo) Median (95% CI)	OS (mo) p^a	RR (%) CR + PR	RR (%) SD + PD	RR (%) p^b
BRCA1 high	54 (54)	2.2 (1.7–2.8)	0.041	6.7 (3.1–7.8)	0.005	6	94	0.034
BRCA1 low	46 (46)	4.0 (2.7–5.8)		14.7 (10.9–23.2)		26	74	
ERCC1 high	55 (55)	2.0 (1.7–2.3)	0.003	5.8 (5.1–6.5)	0.001	7	93	0.013
ERCC1 low	45 (45)	4.2 (3.0–5.4)		15.8 (9.9–21.7)		29	71	
Both low	43 (43)	4.1 (2.2–6.2)	0.002[c]	16.0 (11.4–26.3)	<0.001[c]	30	70	0.006[c]
Both high	45 (45)	2.0 (1.7–2.3)	0.054[d]	5.4 (2.9–7.8)	0.008[d]	4	96	0.035[d]
One high other low	12 (12)	3.4 (2.3–4.3)		8.8 (7.7–11.8)		8	92	0.048[e]

[a] Log-rank p value.
[b] χ^2 p value.
[c] Both low versus both high.
[d] Both low versus one high other low.
[e] Both high versus one high other low.
PFS, progression-free survival; OS, overall survival; RR, response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; CI, confidence interval.

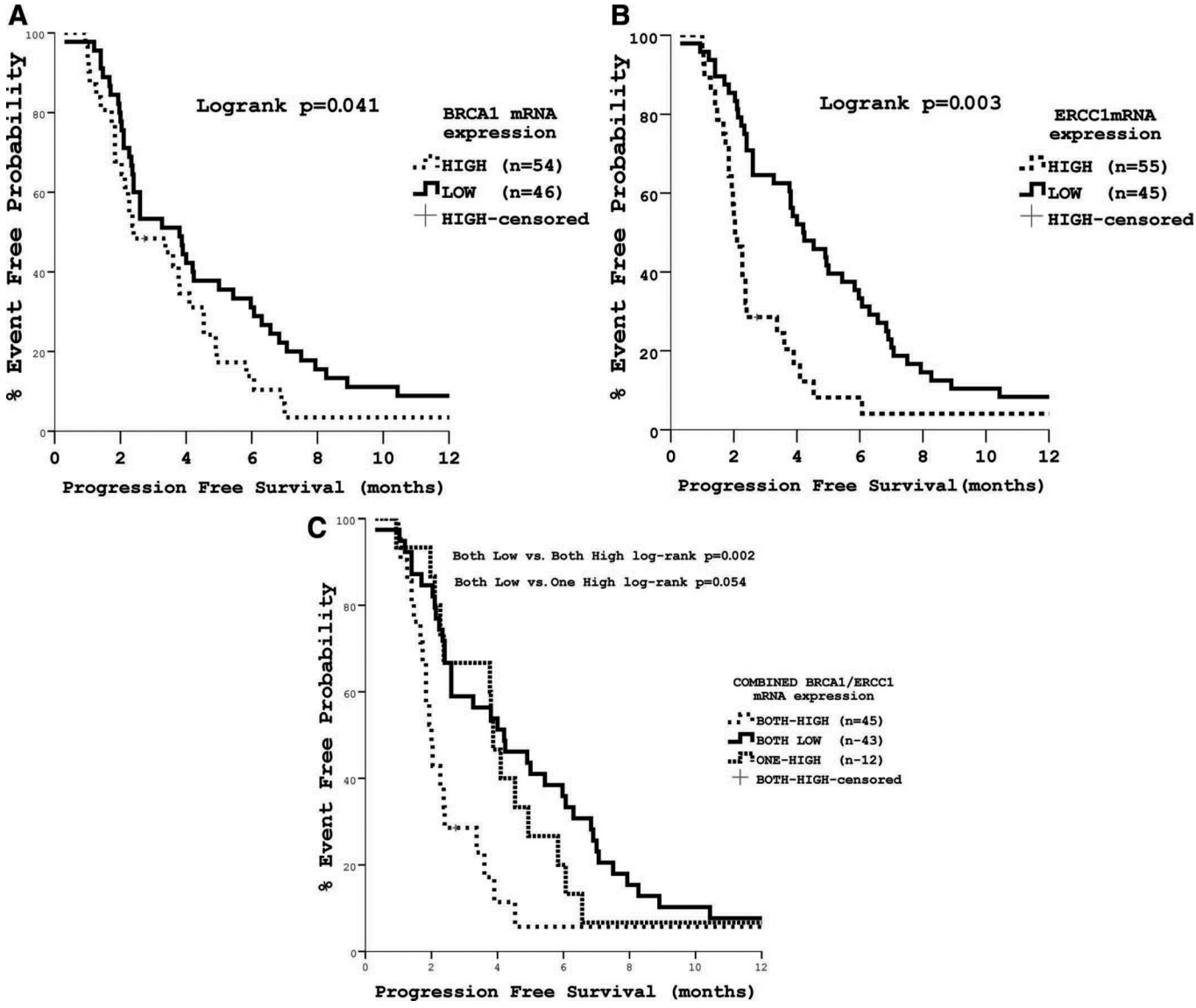

FIGURE 2. Progression-free survival (PFS) according to *BRCA1* (*A*), *ERCC1* (*B*), and *BRCA1–ERCC1* combined (*Cc* mRNA expression levels. Lower levels of BRCA1 (*A*), *ERCC1* (*B*), and both genes (*C*) were associated with significantly decreased PFS (for more details see Table 2).

first-line treatment (HR: 1.31, 95% CI: 1.26–1.98; $p = 0.036$) were significantly associated with decreased PFS, whereas age more than 70 years ($p = 0.37$), gender ($p = 0.46$), and histology ($p = 0.32$) were not significantly associated with the PFS (Table 3). Similarly, high *BRCA1* (HR: 2.15, 95% CI: 1.21–3.80; $p = 0.008$) and *ERCC1* (HR: 3.46, 95% CI: 1.89–6.32; $p < 0.001$) mRNA expression level were associated with decreased mOS. In addition, PS of 1 (HR: 1.52, 95% CI: 1.02–2.38; $p = 0.044$) and no response to prior first-line chemotherapy (HR: 1.38, 95% CI: 1.14–2.62; $p = 0.041$) were significantly correlated with decreased OS, whereas age ($p = 0.39$), gender ($p = 0.52$), and histology ($p = 0.54$) presented no significant impact on mOS (Table 3). A Cox proportional hazard analysis revealed that mRNA expression levels of *BRCA1* (HR: 1.93, 95% CI: 1.46–1.87; $p = 0.013$) and *ERCC1* (HR: 2.32, 95% CI: 1.36–3.96; $p = 0.002$) emerged as the only independent factors associated with decreased PFS (Table 4). Similarly, *BRCA1* (HR: 1.87, 95% CI: 1.13–2.58; $p = 0.016$) and *ERCC1* (HR: 1.91, 95% CI: 1.25–2.92; $p = 0.003$) mRNA expression levels were revealed as the only independent factors correlated with decreased mOS (Table 4).

DISCUSSION

In the past decade, an overwhelming amount of data has been generated concerning the role of tumor molecular profiling to the chemotherapeutic drug activity. *BRCA1* and *ERCC1*, two major components of NER and base excision repair pathways, respectively, have been proposed as predictive biomarkers of

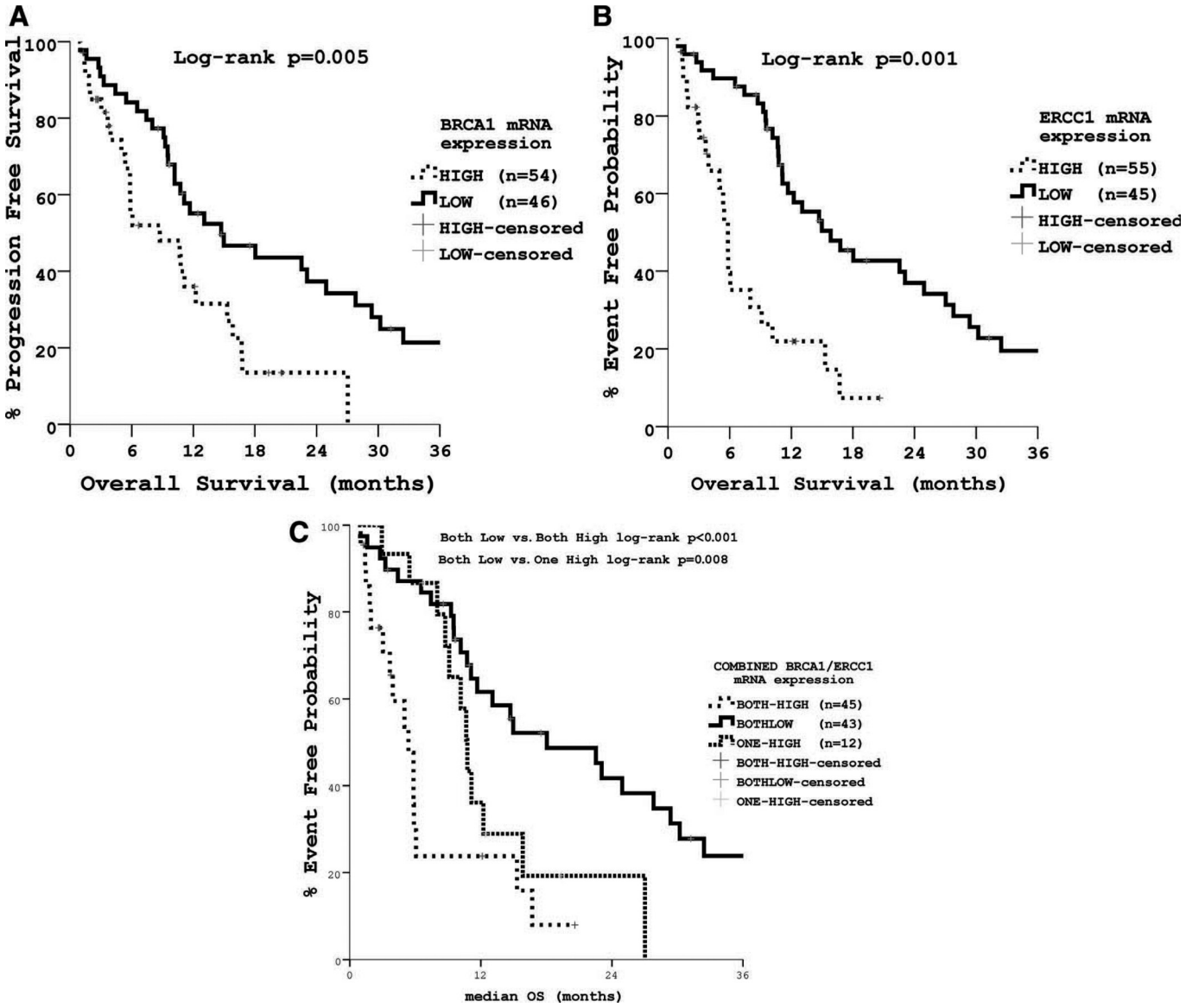

FIGURE 3. Median overall survival (OS) according to *BRCA1* (*A*), *ERCC1* (*B*), and *BRCA1–ERCC1* combined expression (*C*) mRNA expression levels. Lower levels of BRCA1 (*A*), *ERCC1* (*B*), and both genes (*C*) were associated with significantly decreased progression-free survival (for more details see Table 2).

the treatment of NSCLC patients with platinum-based chemotherapy in the adjuvant or first-line setting.[18–20,30,33,38] In this study, the role of *BRCA1* and *ERCC1* mRNA expression levels on the outcome of NSCLC patients received second-line platinum-based therapy was investigated. On the basis of our data, patients with low *BRCA1* and/or *ERCC1* mRNA expression levels attained a significantly higher RR and longer PFS and OS compared with those with high expression level of either gene. Moreover, the multivariate analysis revealed that *BRCA1* and *ERCC1* expression levels were independent factors for PFS and OS. These results confirm our earlier findings for the predictive role of *BRCA1* in a smaller cohort of 31 NSCLC patients treated with cisplatin-based combinations in the second-line setting.[33] Taking together the results of the two studies support the previous findings for the differential predictive role of *BRCA1* expression level concerning the response to cisplatin and antimicrotubule agents,[27] and this hypothesis is currently being tested in a prospective randomized trial (BREC http://clinicaltrials.gov/ct2/show/NCT00617656?term=BREC&rank=1). In support of the clinical finding, in vitro studies using breast cancer cell lines have shown that decreased tumoral BRCA1 mRNA levels increase the sensitivity to cisplatin and resistance to antimicrotubule drugs; accordingly, low BRCA1 expression level was associated with cisplatin efficacy in a variety of solid tumors.[28,30,39] Overexpression of ERCC1 and BRCA1 seems to be oncogene driven,[40] and there is no clear explanation why these two as well as RRM1 are found upregulated especially in squamous cell lung cancer. In this study, the observed positive correlation ($p = 0.002$) between the

TABLE 3. Univariate Analysis for PFS and OS

	Hazard Ratio	95% CI	*p*
PFS			
BRCA1 expression level (high vs. low)	1.61	1.20–2.83	0.002
ERCC1 expression level (high vs. low)	1.82	1.37–3.13	0.001
Response to first-line treatment (SD + PD vs. CR + PR)	1.31	1.26–1.98	0.036
PS (1 vs. 0)	1.56	1.29–2.58	0.026
Age (>70 yr vs. ≤70 yr)	1.24	0.79–1.78	0.37
Gender (male vs. female)	1.12	0.86–1.95	0.46
Histology (squamous vs. nonsquamous)	1.22	1.14–1.78	0.32
OS			
BRCA1 expression level (high vs. low)	2.15	1.21–3.80	0.008
ERCC1 expression level (high vs. low)	3.46	1.89–6.32	<0.001
Response to first-line treatment (SD + PD vs. CR + PR)	1.38	1.14–2.62	0.041
PS (1 vs. 0)	1.52	1.02–2.83	0.044
Age (>70 yr vs. ≤70 yr)	1.19	0.81–1.93	0.39
Gender (male vs. female)	1.10	0.82–1.53	0.52
Histology (squamous vs. nonsquamous)	1.13	0.83–1.24	0.54

PFS, progression-free survival; OS, overall survival; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; CI, confidence interval; PS, performance status.

TABLE 4. Multivariate Analysis for Time to Tumor Progression and Overall Survival

	Hazard Ratio	95% CI	*p*
PFS			
Response to first-line treatment (SD + PD vs. CR + PR)	1.21	0.54–1.76	0.84
BRCA1 expression level (high vs. low)	1.93	1.46–2.87	0.013
ERCC1 expression level (high vs. low)	2.32	1.36–3.96	0.002
PS (1 vs. 0)	1.19	0.62–1.76	0.67
OS			
Response to first-line treatment (SD + PD vs. CR + PR)	1.15	0.65–2.21	0.41
BRCA1 expression level (high vs. low)	1.87	1.13–2.58	0.016
ERCC1 expression level (high vs. low)	1.91	1.25–2.92	0.003
PS (1 vs. 0)	1.21	0.72–1.96	0.44

PFS, progression-free survival; OS, overall survival; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; PS, performance status; CI, confidence interval.

expression levels of BRCA1 and ERCC1 and the clinical outcome after second-line platinum based-chemotherapy is in agreement with in vitro and in vivo studies.[40] Furthermore, in the same line with previous reports was the finding that the expression level of both genes is significantly higher in squamous cell carcinoma (BRCA1, $p = 0.001$; ERCC1, $p = 0.03$) compared with adenocarcinoma and other histological types.[18,19,40] In addition, patients with low expression level of both BRCA1 and ERCC1 genes obtained the maximum benefit from cisplatin, with a significantly higher RR, PFS, and OS.

Despite clinically meaningful activity of pemetrexed and docetaxel in NSCLC patients,[7,8] the results from several phase III trials show that second-line treatment efficacy has reached a plateau, necessitating new directions for NSCLC treatment decisions. The option of using potential molecular predictors of response and survival in the basis of a customized treatment seems now to be reasonable. The data presented here imply that other known predictive biomarkers could be tested in NSCLC patients receiving second-line therapy. For example, high thymidylate synthetase levels, which is the main target of pemetrexed,[41] were associated with reduced sensitivity of pemetrexed in cell lines.[42,43] On the basis of the results of this study, an analysis for the predictive significance of thymidylate synthetase expression level to pemetrexed efficacy in the second-line treatment may be a reasonable approach for a subsequent study.[2,44]

In this study, it is noteworthy that these results were obtained with the analysis of the initial biopsy of the primary tumor at the time of diagnosis. Despite the fact that differences in *KRAS* and/or endothelial growth factor receptor mutation status between primary tumor and metastasis have been reported in approximately 25% of the cases,[45] there is a lack of data regarding *ERCC1* and *BRCA1* expression levels between primary tumor and metastatic sites. Also, not much is known regarding the changes in the expression profile after the exposure to chemotherapeutic agents in vivo. In any case, the results of this study indicate that potentially *ERCC1* and *BRCA1* expression levels in the primary tumor at the time of diagnosis could be used for customization of the second-line treatment of patients with NSCLC.

The results of this study should be interpreted with caution. The study was conducted retrospectively in a relatively small cohort of patients. Therefore, these findings should be confirmed in a larger independent cohort of patients preferable in the context of a randomized trial in which cisplatin-based chemotherapy was used only in one arm. Ideally, the hypothesis that gene expression of the primary tumor could predict the efficacy of second-line chemotherapy could be tested with a rebiopsy of the metastatic tumor at the time of progression. This strategy could allow the comparison between the expression values before and after treatment exposure and their predictive significance and could provide important information for the mechanisms of acquired resistance to the initial treatment.

In conclusion, although our findings should be interpreted cautiously according to the limitations described earlier, our results suggest that the assessment of *BRCA1* and *ERCC1* mRNA expression levels can be used to select NSCLC patients in that they would have benefit from platinum-based combinations in the second-line treatment.

Further studies in a larger cohort of patients and other molecular markers are warranted to confirm these results.

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